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Research review paper

Verbascoside — A review of its occurrence, (bio)synthesis and pharmacological significance

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ABSTRACT

Phenylethanoid glycosides are naturally occurring water-soluble compounds with remarkable biological properties that are widely distributed in the plant kingdom. Verbascoside is a phenylethanoid glycoside that was first isolated from mullein but is also found in several other plant species. It has also been produced by *in vitro* plant culture systems, including genetically transformed roots (so-called 'hairy roots'). Verbascoside is hydrophilic in nature and possesses pharmacologically beneficial activities for human health, including antioxidant, anti-inflammatory and antineoplastic properties in addition to numerous wound-healing and neuroprotective properties. Recent advances with regard to the distribution, (bio)synthesis and bioproduction of verbascoside are summarised in this review. We also discuss its prominent pharmacological properties and outline future perspectives for its potential application.

Contents

Treasure from the garden: the discovery of verbascoside, and its occurrence and distribution

Phenylethanoid glycosides are naturally occurring water-soluble compounds that are widely distributed in the plant kingdom, most of which are isolated from medicinal plants. Structurally, they are characterised by cinnamic acid (C_6–C_3) and hydroxyphenylethyl (C_6–C_2) moieties that are attached to a β-glucopyranose (apiose, galactose, rhamnose, xylose, *etc.*) *via* a glycosidic bond (Dembitsky, 2005; Jimenez and Riguera, 1994). In recent years, interest has been growing regarding aromatic compounds, and phenylethanoid glycosides in particular, because of the significantly increasing volume of literature describing their evident role in the prevention and treatment of various human diseases and disorders.

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There are conflicting reports in the literature regarding the designation of verbascoside. In 1963, scientists from Italy reported the isolation of a phenylethanoid glycoside from mullein (*Verbascum sinuatum* L.; Scrophulariaceae), which they called verbascoside (Fig. 1; Scarpati and Monache, 1963). However, no data describing its structural elucidation was given. Several years later, the same compound was isolated from flowers of the common lilac (*Syringa vulgaris*, Oleaceae), and the structure was determined to be 2-(3, 4-dihydroxyphenyl)ethyl-1-*O*-α-L-rhamnopyranosyl-(1→3)-(4-*O*-*E*-caffeoyl)-β-D-glucopyranoside, which the authors named acteoside (Birkofer et al., 1968). Twenty years after the first report on verbascoside, Sakurai and Kato (1983) reported the isolation of a new phenylethanoid glycoside from the glory tree (*Clerodendron trichotomum* Thunb, Lamiaceae) that was named kusaginin by the authors. While isolating verbascoside from the greater broomrape (*Orobanche rapum-genistae*, Orobanchaceae), Andary et al. (1982) discovered that it is identical to the previously reported acteoside and recommended that this later name be no longer used. Currently, 50 years after the discovery of verbascoside, confusion still exists with regard to its name to a certain degree. A search of the Scopus database (accessed January 2014) pulled out 543 available hits under 'verbascoside' and 844 hits under "acteoside", while a search using the paired terms 'verbascoside AND acteoside' returned only 326 articles. To prevent any further confusion, we highly recommend that either verbascoside or acteoside be primarily used; however, the other name should also be supplied in the article's abstract or keyword list.

Verbascoside is among the most widespread of the disaccharide caffeoyl esters. To date, verbascoside has been mainly detected in the *Verbascum* species (Alipieva et al., 2014) but has also been found in more than 200 plant species (Deepak et al., 1999; Schlauer et al., 2004; Taskova et al., 2005) belonging to 23 plant families (Fig. 2) in addition to others that were recently reported, such as *Buddleja brasiliensis* (Filho et al., 2012), *Striga asiatica* (Huang et al., 2013), *Olea europea* (Quirantes-Piné et al., 2013b), *Paulownia tomentosa* var. *tomentosa* (Si et al., 2013), *Lippia javanica*, *Lantana camara* (Oyourou et al., 2013), and *Lippia citriodora* (Bilia et al., 2008; Funes et al., 2009; Quirantes-Piné et al., 2009).

Verbascoside has been detected in both underground (*e.g.*, primary and secondary roots) and above-ground (*e.g.*, stems, leaves and flowers) parts of plants but at widely varying levels. For example, the roots of *Sideritis trojana* accumulate verbascoside at 0.002% (Kirmizibekmez et al., 2012), while in the aerial parts of *Verbascum xanthophoeniceum* its concentration is much higher (0.25%; Georgiev et al., 2011a). In a recent nuclear magnetic resonance (NMR)-based metabolomics study, it was found that verbascoside content varies within the plant species of the same genus; from 0.2% in *V. phoeniceum* up to 3% in *Verbascum nigrum* (Georgiev et al., 2011b). On the other hand, verbascoside content in roots (0.9%) and inflorescences (0.8%) was comparable (Gómez-Aguirre et al., 2012). Another potentially valuable source of verbascoside includes industrial by-products. For example, olive mill waste that was obtained as a by-product from the processing of olive fruit was found to contain abundant amounts of verbascoside in addition to several other phenolic compounds (*e.g.*, oleuropein, hydroxytyrosol, caffeic acid and some flavonoids; Obied et al., 2007). Verbascoside was reported to be abundant in olive mill wastewater (De Marco et al., 2007). Although more detailed research is needed, olive mill wastewater could be considered to be a potential source of verbascoside (and concomitant bioactive molecules) that are suitable for applications involving dietary supplements and food (Cardinali et al., 2012).

Biological effects of verbascoside underlying its potential clinical utility

Traditionally, plants with high concentrations of verbascoside have been used in folk medicine to treat inflammation and microbial infections (Georgiev et al., 2012). Therefore, investigations into its anti-microbial and anti-fungal activities have been conducted over the course of many years. In general, these studies may be regarded as purely observational because they lack any mechanistic approaches (Arruda et al., 2011; Avila et al., 1999; Pendota et al., 2013). Recent studies of seven compounds that were isolated from the *Lippia* species have confirmed the very high anti-*Cryptococcus neoformans* activities of verbascoside, which the authors interpreted to be promising for new selective anti-fungal verbascoside-containing drugs (Funari et al., 2012). The combination of anti-bacterial, anti-inflammatory and anti-androgen effects of pure verbascoside and plant extracts with their high concentrations (*Camellia sinensis* and *Commiphora mukul*) show promise in the development of pharmacological treatments for acne vulgaris (Azimi et al., 2012).

Mechanisms of anti-inflammatory effects of verbascoside on skin, endothelium, intestine and lungs

Early reports describing the anti-inflammatory effects of verbascoside have focused on the inhibition of histamine and arachidonic acid release from mast cells (Lee et al., 2006a). The inhibitory activity of verbascoside depends on the presence of Ca^{2+} and correlates with the verbascoside-associated inhibition of phospholipase A2 (Song et al., 2012). Mechanistic studies have been carried out recently, revealing that verbascoside down-regulates Ca^{2+}-dependent MAPK signalling in basophilic cells (Motojima et al., 2013). It appears to become involved in the inhibition of type I allergies. Glycosylated phenylethanoids, which are constituents of *Anisomeles indica*, exhibit profound anti-inflammatory activities towards macrophages that are stimulated by LPS and IFN-γ. In particular, 40 μM verbascoside has been shown to strongly inhibit NO, TNF-α and IL-12 production (Rao et al., 2009). The anti-inflammatory and anti-irritant activities of verbascoside from *Kigelia africana* have been attributed to its ability to inhibit inducible nitric oxide synthase (iNOS) and NO release from macrophages as stimulated by bacterial lipopolysaccharides (Picerno et al., 2005). The inhibition of iNOS *in vitro* and *in vivo* by a water-soluble extract of *Wendita calysina* leaves was attributed to

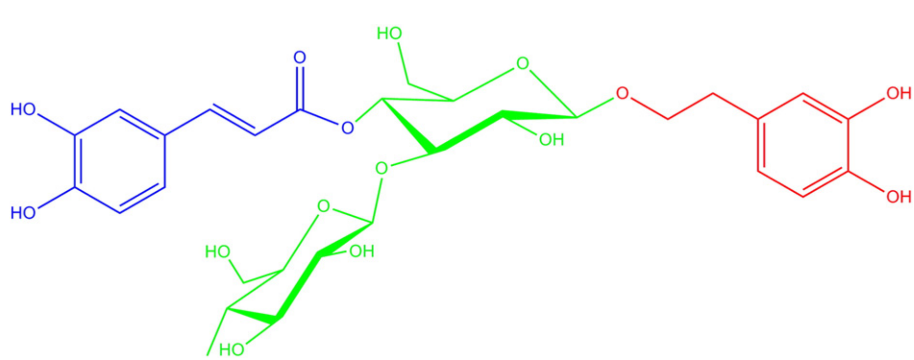

Fig. 1. Chemical structure of verbascoside. Phenylethanoid (in red) and caffeic acid (in blue) moieties attached to α-rhamnopyranosyl-β-glucopyranose (in green).

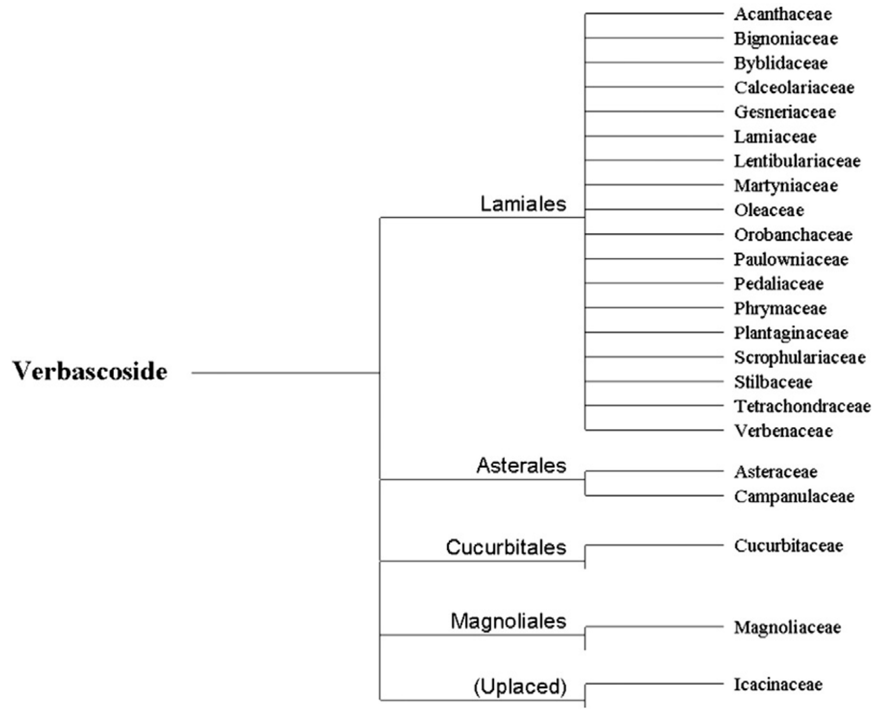

Fig. 2. Distribution of verbascoside in the plant kingdom (modified after Schlauer et al., 2004).

verbascoside as well (Marzocco et al., 2007). In parallel with the iNOS/NO inhibition, verbascoside induced heme oxygenase 1, while it suppressed high mobility group box 1 release in the *in vitro* and *in vivo* experiments, and these effects were abolished by Nrf2 silencing (Seo et al., 2013). This anti-iNOS mechanism was closely connected to the down-regulation of two transcription factors (NFκB and AP-1) by verbascoside (Lee et al., 2005). Activator protein 1 (AP-1) is a transcriptional regulator of cytokine expression in bone, skin, and immune cells and an important modulator of inflammatory processes in chronic inflammatory diseases, such as rheumatoid and psoriatic arthritis, psoriasis and Crohn's disease (Zenz et al., 2008). The essential roles of AP-1 proteins in the control of cell proliferation, neoplastic transformation and apoptosis have also been identified (Shaulian and Karin, 2001). With the tremendous recent advancements in the molecular understanding of the pathogenesis of these human pathologies, AP-1 has become one of the new targets for therapeutic applications. Notably, verbascoside and its glycosylated derivative teupolioside are the only established examples of the effective combined inhibition of the expression and DNA binding of both pro-inflammatory transcriptional factors, AP-1 and NFκB (Pastore et al., 2009, 2012a). Other plant polyphenols (resveratrol, quercetin, *etc.*) that have been studied under the same experimental settings have been shown to be selective inhibitors of NFκB. The suppression of both these transcription factors by phenylethanoids resulted in the much more effective inhibition of pro-inflammatory chemokine IL-8 compared with other polyphenols (Pastore et al., 2009; Potapovich et al., 2011; Georgiev et al., 2012). The chemokine attracts granulocytes to the inflammatory loci and induces cell proliferation, which is characteristic of inflamed tissues (Pastore and Korkina, 2010).

In the mechanistic study of the effects of verbascoside on human myelomonocytic leukaemia cells that have been exposed to pro-inflammatory stimuli, such as LPS and interferon gamma, Speranza et al. (2010) reported that its anti-inflammatory activities are dependent on the inhibition of both the expression and activity of iNOS, the inhibition of intracellular superoxide anion production and the suppression of SOD, catalase, and glutathione peroxidase at the post-translational level.

Large amounts of data exist in support of the regulatory role of verbascoside in vascular inflammation that is induced by oxidised low density lipoproteins (oxLDL, which are models of chronic inflammation leading to atherosclerosis) and induced by bacterial lipopolysaccharides (LPS, which is a model of intravascular septic inflammation; Kostyuk et al., 2011). Verbascoside selectively prevented LDL oxidation by peroxynitrite and down-regulated genes that are associated with oxidative stress and inflammation in response to oxLDL (*e.g.*, VCAM1, ICAM1, IL-8, IFN-γ, SOD2, iNOS, and NOX1). Additionally, it was completely ineffective towards LPS-induced inflammation. Four phenylethanoid glycosides, including verbascoside, protected endothelial cells against oxLDL-induced cytotoxicity (Martin-Nizard et al., 2003) that was derived from oxidative stress (Chiou et al., 2004).

Beyond its well-investigated anti-inflammatory activities *via* NFκB and MAPKs, verbascoside can affect gene expression through a variety of other signalling pathways, whose impacts on the expression of inflammatory mediators have been investigated only marginally to date. In particular, because it is an aromatic hydrocarbon, verbascoside binds the aryl hydrocarbon receptor (AhR) transcription factor, which thus contribute to the transcription of numerous detoxification genes coding for phase I and II metabolising enzymes, particularly the cytochrome P450 CYP1 subfamily, Nrf2, glutathione S-transferase (GST) and antioxidant enzymes. As a result, verbascoside inhibits downstream pro-inflammatory cytokines and growth factors (Korkina et al., 2011; Potapovich et al., 2011).

Bioavailability and metabolism of dietary and topically applied verbascoside

In general, the systemic effects of plant polyphenols mainly depend on their bioavailabilities through the gastrointestinal barrier. Highly disparate data describing the *in vitro* and *in vivo* biological activities of dietary polyphenols appear to reflect their fates in the gut, including relatively poor bioavailabilities and rapid rates of metabolism and excretion (Martin and Appel, 2010). While numerous investigations into their bioactivities in cell cultures have revealed that the optimal range of concentrations for *in planta* polyphenolic aglycones and sugar conjugates are within the μM to low mM range, following ingestion, they appear in the circulation as phase II metabolites, and their plasma levels rarely exceed nM concentrations (Del Rio et al., 2013). Substantial

portions of both parent polyphenols and their metabolites reach the colon, where they are digested by the local microbiota to small phenolic acid and aromatic catabolites. They are easily absorbed into the circulatory system and subsequently travel to target tissues, where they exert their protective and/or curative effects. The recent comprehensive review that was conducted by Del Rio et al. (2013) provided data on the bioavailability, metabolism and pharmacokinetics of several dietary polyphenolics with extensively reported health properties, such as quercetin, curcumin and resveratrol. Unfortunately, we failed to find publications on the bioavailability and pharmacokinetics of verbascoside in humans. A few animal studies on the subject confirmed that it is quickly absorbed (its highest plasma concentration was reached after approximately 15 min) and eliminated from rats (Li et al., 2014). Further, its oral bioavailability was as low as 0.12% (Wu et al., 2006). Verbascoside was distributed evenly in all of the brain compartments that were studied within nM concentrations. Thorough analytical assessment of the plasma levels of verbascoside and its metabolites has been carried out after feeding rats with *Lippia citriodora* (lemon verbena) extracts (Quirantes-Piné et al., 2013a). The main plasma compounds included verbascoside and isoverbascoside (approximately 80 and 60 ng/mL, respectively), while the minor metabolites were hydroxytyrosol, caffeic acid, ferulic acid and its glucoronide, and homoprotocatechuic acids.

Because the physicochemical characteristics of polyphenols, such as their molecular sizes, degrees of polymerisation and solubilities are key determinants for their absorption in the digestive tract and further metabolism and appearance in the circulatory system (Martin and Appel, 2010), one can assume that verbascoside would be easily absorbed similar to catechins, flavanones and quercetin glycosides. Thus, human studies on verbascoside bioavailability, metabolism and pharmacokinetics may shed light on the mechanisms underlying its systemic health effects.

The bioavailability and biotransformation of plant polyphenols in the skin upon topical application are completely different from the processes that take place upon ingestion. The highly effective physical barrier properties of the skin make the task of foreign molecule delivery to the skin itself and to internal tissues extremely complicated. In fact, all exogenous substances with low lipophilicities, such as verbascoside, have limited capacities for skin permeation (Korkina et al., 2009). Skin permeation and the transdermal delivery of verbascoside can be increased substantially by its inclusion into liposomes or lipogels (Sinico et al., 2008). The cutaneous chemical barrier consists of numerous phase I and phase II metabolic enzymes and non-enzymatic molecules that are capable of reacting with and facilitating the metabolism of low-molecular-weight foreign substances. Verbascoside was able of acting the aryl hydrocarbon receptor that is connected with the downstream cytochrome P450 CYP subfamilies; for example, CYP1A1 and CYP1B1 in addition to glutathione peroxidase (phase I)- and glutathione-S-transferase (phase II)-metabolising enzymes in human keratinocytes (Pastore et al., 2012a, 2013; Potapovich et al., 2011).

Evidence of systemic anti-inflammatory and indirect antioxidant effects of verbascoside and its metabolites in animal experiments and human clinical studies

Although verbascoside has been shown to possess rather modest direct reactive oxygen species scavenging activities compared with several other plant polyphenols in model systems (Pastore et al., 2012a), its metabolites were shown to significantly enhance the activities of major antioxidant enzymes (catalase, glutathione peroxidase and glutathione reductase) while suppressing pro-oxidant- and inflammation-related myeloperoxidase in the circulating lymphocytes, erythrocytes and neutrophils of rats following the ingestion of lemon verbena extract (Quirantes-Piné et al., 2013a). The authors suggested that verbascoside affects the redox enzymes at the post-transcriptional level. Additionally, several publications have provided clear evidence that verbascoside could induce the gene transcription of antioxidant enzymes *via* an Nrf2-dependent mechanism (Kostyuk et al., 2011, 2013; Pastore et al., 2012a,b).

In the experimental model of intestinal inflammation that is induced by dextran sulphate, which resembles immune-mediated inflammatory bowel disease in humans, the systemic administration of verbascoside that was isolated from *Plantago lanceolata* L. substantially improved the histological patterns and clinical symptoms of colitis, down-regulated pro-inflammatory IFN-γ secretion and inhibited the NADPH-oxidase-connected oxidative burst (indirect antioxidant effect) in the intestinal macrophages (Hausmann et al., 2007; Lenoir et al., 2011). Further studies with verbascoside from plant cell cultures of *Syringa vulgaris* L. have confirmed its protective and curative effects through reduced NFκB activation. As a result, NFκB-dependent cellular events, such as metalloproteinase (MMP2 and MMP9) activation, inducible nitric oxide synthase and poly(ADP ribose) up-regulation and adhesion factor expression have been attenuated (Mazzon et al., 2009). It appears that peroxisome proliferator-activated receptor-α (PPAR-α) significantly contributes to the anti-inflammatory effects of verbascoside in experimental colitis that is induced by dinitrobenzene sulfonic acid because in mice with genetically abolished PPAR-α, such effects were not observed (Esposito et al., 2010a). Phenylethanoid glycosides from *Castilleja tenuiflora* Benth (isoverbascoside and verbascoside) provided significant gastric protection in the *in vivo* model of acute gastric ulcers and in a mouse ear oedema model. The anti-inflammatory effects were comparable with that of dexamethasone (Sanchez et al., 2013). Dietary intervention with verbascoside has been shown to prevent intestinal damage and protein nitrosylation in swine (Di Giancamillo et al., 2013). Its oral administration from plant cell cultures also suppressed ligature-induced periodontitis, which was assessed by the inhibition of several inflammatory markers, such as myeloperoxidase, NFκB and iNOS expression, nitration and lipid peroxidation end products (Paola et al., 2011).

The bio-guided isolation of wound healing glycosides from the flowers of *Verbascum mucrotanum* Lamm resulted in the conclusion that orally applied verbascoside possesses the highest combinatory (wound healing, anti-inflammatory and anti-nociceptive) activity compared with other glycosides that were isolated from the plant. Additionally, verbascoside did not exhibit any acute toxicity or gastric damage (Akdemir et al., 2011). The strong wound healing and anti-inflammatory effects of topically applied verbascoside have been previously described in animal models of excisions and scarification wounds (Korkina et al., 2007).

There are several human interventional studies involving dietary verbena and lemon verbena extracts containing large quantities of phenylpropanoids, a major part of which is verbascoside. Thus, according to analytical analyses that were carried out by Bilia et al. (2008), the total concentrations of phenylpropanoids in aqueous solutions of plant leaves were 20–150 mg/g dry weight, 97% of which was verbascoside, which possessed remarkable antioxidant properties in the *in vitro* experiments. Another analytical study confirmed the presence of verbascoside and isoverbascoside as major phenolic components of lemon verbena (Quirantes-Piné et al., 2009). A randomised, double-blind, placebo-controlled study of the efficacy of lemon verbena extract in combination with omega-3 fatty acids in joint management has been carried out (Caturla et al., 2011). The extract reduced the symptoms of pain and stiffness and improved physical functioning significantly in subjects with joint discomfort. As a secondary outcome, the lemon verbena extract showed strong antioxidant properties. The extract significantly protected blood components from physical exercise-associated oxidative stress by the modulation of GSH-reductase activity, which has been previously observed in a double-blind human study (Carrera-Quintanar et al., 2012). Antioxidant supplementation with lemon verbena extract has been shown to protect neutrophils against oxidative damage that was induced by chronic exercise, decreased myeloperoxidase activity and muscular damage without affecting either

the immune or antioxidant adaptation to exercise (Funes et al., 2011). Moreover, a verbascoside-containing extract was shown to enhance glutathione-dependent enzymes and superoxide dismutase in circulating blood cells of female swimmers while decreasing the plasma levels of sex hormones (Mestre-Alfaro et al., 2011). These data that were obtained from the very first human studies, which were mainly pilot studies, provided some preliminary evidence on the positive systemic effects of verbascoside. However, the mechanisms underlying these effects remain to be elucidated and further clinical research is necessary.

Verbascoside in protection from UV irradiation

For the human skin, UV radiation represents a dramatic environmental stimulus underlying numerous pathological manifestations that eventually lead to premature ageing and cancer (Korkina and Pastore, 2009; Korkina et al., 2009; Kostyuk et al., 2013; Pastore et al., 2012b). These deleterious effects are strictly associated with irreversible damage to cell macromolecules, including DNA and proteins, as a consequence of the persistent overproduction of reactive oxygen species (ROS) and depletion of endogenous antioxidants. It is now generally accepted that UV radiation induces chronic inflammation in the skin, which in its turn aggravates persistent local free radical/antioxidant imbalance. UV interacts with extremely complex cutaneous compartments at different levels. First, it induces photochemical reactions in the outermost superficial skin surface lipids (SSL). Then, it photochemically modifies components of non-viable keratinocytes and intercellular lipids of the stratum corneum, and finally, directly or through photochemical mediators it reaches the viable layers of epidermis and the underlying dermal compartments. Consequently, the identification of natural substances for the effective photoprotection and suppression of inflammatory reactions in the skin is presently a major concern in investigative dermatology. Theoretically, plant-derived secondary metabolites for skin photo-protection should be photo-stable and not promote photochemical reactions in skin components. They could modify skin-UV interactions at different crucial points as follows: (a) absorbing UVA + UVB (screen action); (b) interrupting UV-induced free radical-driven reactions in skin components (scavenging and direct antioxidant chain-breaking activities); (c) protecting endogenous SSL antioxidants, such as α-tocopherol, coenzyme Q10 and squalene (antioxidant rescue activities); (d) inducing endogenous antioxidant systems in keratinocytes (indirect antioxidant activities); (e) attenuating the inflammatory response in keratinocytes (anti-inflammatory activities); and (f) modulating excessive metabolic and proliferative stress responses (anti-stress action). The panel of methods allowing for the comparison of all of the above-mentioned activities of several plant polyphenols (resveratrol, polydatin, quercetin, rutin and verbascoside) has been applied by Kostyuk et al. (2013). On the grounds of the data that were obtained, the authors concluded that verbascoside was the best candidate for topical photo-protection and consequently for the chemoprevention of UV-induced non-melanoma skin cancers. The molecular features of effective photo-protection by verbascoside are presented in Fig. 3.

In general, cellular UV-induced processes can be divided into at least two phases. The first one consists of photophysical and photochemical events occurring during and immediately following exposure to UV. These events trigger a number of molecular, biochemical and cellular alterations that are characteristic for the second phase. For example, during the second phase, numerous signal transduction pathways are stimulated in keratinocytes that lead to the activation of UV-sensitive membrane (EGFR) and nuclear (AhR) receptors (Pastore et al., 2012a, b; Potapovich et al., 2011). Thus subsequent initiation of intracellular signalling results in the modulation of underlying genes and *de novo* synthesis of inflammatory cytokines, chemokines and adhesion molecules, thus forming a pro-inflammatory pattern characteristic of UV-responses. Another characteristic feature is the stimulation of certain metabolic processes that are mediated by AhR (Pastore et al., 2012a,b) and excessive intracellular NO production by inducible NOS (Korkina and Pastore, 2009).

Recent data has strongly suggested that verbascoside is particularly effective in the inhibition of the second remote phase of inflammatory and metabolic responses to solar UV irradiation in human keratinocytes (Potapovich et al., 2013). The delayed protective effects of hydrophilic verbascoside have been explained in terms of its low permeability through the lipid bilayer of skin cell membranes. Indeed, a physicochemical study of the interactions of verbascoside with phospholipid model membranes (Funes et al., 2009) has revealed the localisation of the phenylpropanoid molecules in the upper layer of the phosphatidylglycerol membrane at the phospholipid/water interface. Another example of a remote second-phase response to UV in the skin is increased melanogenesis. Verbascoside was shown to effectively inhibit melanin production, which is induced by UV and α-melanocyte-stimulating hormone in melanoma cells *in vitro* by the down-regulation of tyrosinase, which is a key enzyme that is involved in melanin synthesis (Muñoz et al., 2013; Son et al., 2011). Additionally, tyrosinase-connected proteins also inhibit its production while activating ERK phosphorylation (Son et al., 2011) and inactivating adenylate cyclase *via* α-melanocyte-stimulating hormone pathway (Song and Sim, 2009).

Plant-derived polyphenols, such as verbascoside, typically absorb UV light within the wavelength range of 300–400 nm, and hence, they are widely employed as effective UVA–UVB screening molecules. To distinguish UV absorption from the other possible photoprotective properties of verbascoside, the model of UVC irradiation of human keratinocytes was used (Kostyuk et al., 2008; Pastore et al., 2009). Again, verbascoside effectively protected cells from UVC-induced necrosis, which clearly indicates that free radical scavenging was employed in the photo-protection process.

Unfortunately, verbascoside and similar phenylethanoid glycosides are generally water-soluble molecules; therefore their penetration through highly hydrophobic epidermal layers is limited. In an *ex vivo* model of freshly excised porcine skin, verbascoside concentrations in deeper skin layers reached approximately 2 nM at 24 h after application (Ouitas and Heard, 2009). Most likely, due to its slow penetration into viable cell layers of the porcine skin, its inhibitory effects on COX-2 were less evident compared with harpagoside and 8-coumaroylharpagide (Abdelouahab and Heard, 2008). To increase the stability of verbascoside in topical preparations and its bioavailability through cutaneous barriers, it was obtained from *Buddleia davidii* meristemal cell cultures and derivatised to acyl-verbascoside, which possesses a lower hydrophilic profile (Vertuani et al., 2011). Its derivatised form exhibited even higher antioxidant capacities and became more stable in oil/water mixtures compared with the parental molecule.

Cytoprotective effects of verbascoside as basis for its use to treat neurodegenerative diseases and pain

The recent mechanistic studies investigating the cytoprotective properties of verbascoside and several other phenylethanoids have shown that these substances protect human cells not only due to their direct antioxidant and free radical scavenging activities but largely due to the up-regulation of endogenous detoxifying systems (Sgarbossa et al., 2012). The verbascoside, campneoside, forsythoside B, and echinacoside activated Nrf2, which is the nuclear factor regulating protective and antioxidant enzymes, while they inhibited BACH1, which is a repressor of the antioxidant response element, in addition to inducing phase II cytoprotective enzymes, the most prominent of which was heme oxygenase 1 (HO-1). The continuous presence of hydrogen peroxide that is produced in the glucose–glucose oxidase system causes mitochondrial-mediated and caspase-independent growth inhibition and cytotoxicity towards human gingival fibroblasts. Two agents, catalase and verbascoside, have been shown to exhibit cytoprotective

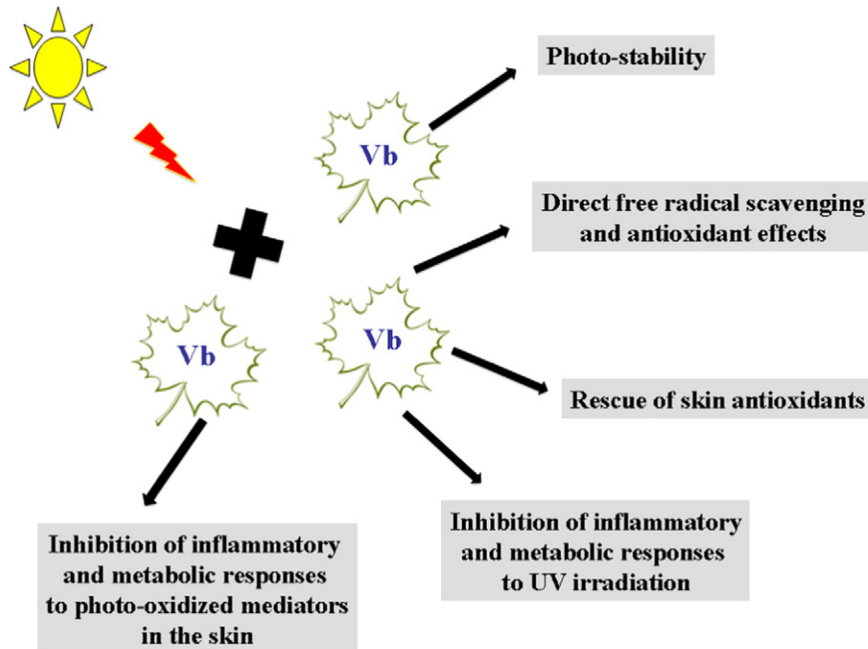

Fig. 3. Molecular characteristics of verbascoside (Vb) underlying its photo-protective activities towards human keratinocytes.

abilities, suppressing the molecular pathways, leading to necrotic cell death (Yu et al., 2012).

The neuroprotective effects of verbascoside have been studied in assorted experimental models. Because neurodegeneration is a complex pathological process, many mechanisms underlie its progression. In one early study (Sheng et al., 2002), the protective effects of verbascoside were examined on 1-methyl-4-phenylpyridinium ion (MPP+)-induced apoptosis and oxidative stress, using pheochromocytoma (PC12) neuronal cells. Neuroprotective effects were determined *via* a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and flow cytometry for apoptosis in addition to the measurement of caspase-3 activity levels and extracellular hydrogen peroxide levels. The findings revealed that verbascoside significantly decreased apoptotic death in PC12 cells, whereas it increased extracellular hydrogen peroxide levels. Consequently, it was concluded to be beneficial for oxidative stress-induced neurodegenerative diseases. In a similar study by the same group (Deng et al., 2008), verbascoside was studied in SH-SY5Y neuronal cells to investigate its protective effects on MPP+-induced injury using several assays, including the MTT assay, the determination of reactive oxygen species (ROS), measurements of caspase-3 activity levels and the expression levels of Bcl-2 and mitochondrial membrane potentials in addition to the flow cytometric assessment of apoptosis. The treatment of the injured neuronal cells with verbascoside at 0.1, 1.0, and 10 mg/L concentrations caused significant improvements in all of the assays that were performed. Verbascoside was able to decrease the range of apoptosis from 38.9% to 29.5% and to deactivate caspase-3, while it led to higher expression levels of the Bcl-2 gene, the diminished accretion of ROS and the MPP+-induced failure of mitochondrial membrane potential in SH-SY5Y cells. As a result, this compound was suggested by the authors to be useful for the treatment of Parkinson's disease (PD). Pu et al. (2003) also showed that verbascoside was able to inhibit MPP-induced neurotoxicity in cerebellar granule neurons by inhibiting apoptosis-related pathways, such as deactivating caspase-3 and the proteolytic poly (ADP-ribose) polymerase (PARP) fragment expression. Wang et al. (2009) demonstrated the protective activities of verbascoside on Aβ(25–35)-induced SH-SY5Y cell injury by decreasing membrane potentials, the modulation of apoptotic signalling *via* Bcl-2, cytochrome c release and the cleavage of caspase-3. In addition, it was tested in a bacterial endotoxin/cytokine LPS/IFN-γ-induced central nervous system inflammation model, and its neuroprotective effects were demonstrated through the modulation of these transcription factors, inhibiting neuronal nitric oxide synthase expression in addition to averting the activation of COX-2, which is a pro-inflammatory enzyme, in glioma cells (Esposito et al., 2010b).

In fact, a number of studies have revealed the effectiveness of verbascoside in experimental models that are related to Alzheimer's disease (AD). It was evaluated for its possible anti-amyloidogenic properties that are relevant to AD, such as its effect on the aggregation of a 42-mer amyloid-β protein (Aβ42), and found that it inhibits Aβ42 aggregation in a dose-dependent manner, suggesting that the catechol moiety of the compound may be responsible for this inhibition (Kurisu et al., 2013).

The inhibition of the aggregation by small molecules seems to represent a promising strategy for the prevention and treatment of AD. Verbascoside and its glycosylated derivatives were extremely potent inhibitors of the aggregation of amyloid proteins at concentrations of <10 μM. Amyloid aggregates provoke a cascade of irreversible oxidative damage to neurons, and verbascoside effectively protected neuronal cells from amyloid-induced injury. The structure–activity relationship suggested that the catechol moieties of phenylethanoids are essential for their anti-amyloid effects. PC12 neuronal cells that were subjected to Aβ-induced cytotoxicity were treated with verbascoside under *in vitro* conditions using HO-1, which is an important enzyme that is involved in neuronal protection, showed neuroprotective activities through the activation of HO-1 and the nuclear translocation of the transcription factor Nrf2 (Wang et al., 2012).

In another work, the inhibitory effects of verbascoside were evaluated against prolyl oligopeptidase (POP), which is a cytosolic serine-type endoprotease that is involved in the pathogenesis of AD. It was found to effectively inhibit POP in a concentration-dependent manner with an IC_{50} value of 1.3 ± 0.2 μM, which is similar to that of the positive control (baicalin, IC_{50} of 12 ± 3 μM; Filho et al., 2012). Additionally, Kahraman et al. (2010) described the moderate inhibitory activities of verbascoside that was isolated from *Verbascum mucronatum* against the cholinesterase enzyme family, which includes the key enzymes that play crucial roles in the pathogenesis of AD. In a similar work by our group, we tested verbascoside and other related compounds (forsythoside B and

Table 1
Antioxidant activity of verbascoside in selected models.

Methods	Activity	References
2,2′-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity	$IC_{50} = 7.18$ μM	Frum et al. (2007)
	$IC_{50} = 8.6$ μM	Hennebelle et al. (2008)
	$IC_{50} = 16.1$ μM	De Souza et al. (2010)
	$EC_{50} = 0.39$ $(M/L_{antiox})/(M/L_{DPPH\bullet})$	Zaabat et al. (2011)
	$IC_{50} = 6.20$ μM	Hung et al. (2012)
	$IC_{50} = 34.7$ μM	Harput et al. (2012)
	$IC_{50} = 5.99$ μM	Si et al. (2013)
	$SC_{50} = 13.1$ μM[a]	Phakeovilay et al. (2013)
Superoxide anion (O_2^-) radical scavenging activity	75% of inhibition at 100 μM	He et al. (2000)
	$IC_{50} = 49.9$ μM (in O_2^- cell free system)	Hennebelle et al. (2008)
Superoxide anion (O_2^-) radical scavenging activity in *Saccharomyces cerevisiae* cells	Presents a 7-fold increase of cellular viability	De Souza et al. (2010)
Nitric oxide (NO) radical scavenging activity	$IC_{50} = 8.06$ μM	Xiong et al. (2000)
Oxygen radical absorbance capacity (ORAC)	$IC_{50} = 24.6$ μM	Aldini et al (2006)
	13 688.7 $ORAC_{FL}$/g	Georgiev et al. (2011a)
	5.0 ORAC unit[b]	Phakeovilay et al. (2013)
Hydroxyl radical averting capacity (HORAC)	1810.2 $HORAC_{FL}$/g	Georgiev et al. (2011a)
Peroxynitrite radical scavenging activity	9.9-Fold total oxidant scavenging capacity (TOSC) of Trolox TOSC value% = 37.02%	Tai et al. (2009)
Ferric-reducing antioxidant power (FRAP)	1.104 (at 0.4 mM)[c]	Kahraman et al. (2010)
	2.602 (at 1.6 mM)[c]	Georgiev et al. (2011a)
H_2O_2 Scavenging ability	$IC_{50} = 13.4$ μM	Hung et al. (2012)
Trolox equivalent antioxidant capacity (TEAC) assay	1.03 ± 0.15 mM (H_2O); 1.28 ± 0.11 mM (EtOH)	Funes et al. (2009)
	1.030 mg/mL	Kirmizibekmez et al. (2012)
Inhibition of lipid peroxidation by electron spin resonance (ESR) technique	Decreases the concentration of oxygen free radicals ($P < 0.05$) and the level of lipid peroxidation ($P < 0.05$) in muscle	Li et al. (1999)
Free radical-induced hemolysis of red blood cells (RBC)	70% of hemolysis inhibition at 30 μM	He et al. (2000)
Cu^{2+}-medicated oxidation of human low-density lipoprotein (LDL)	$ED_{50} = 1.0$ μM	Seidel et al. (2000)
	$IC_{50} = 5.0$ μM	Wong et al. (2001)
Scavenging activity of 2′-deoxyadenosine-5′-monophosphate acid (dAMP) or 2′-deoxyguanosine-5′-monophosphate acid (dGMP) (the oxidised OH adducts)	The electron transfer rate constants of verbascoside with dAMP and dGMP were 5.3×10^8 and 20.2×10^8 L/M/s, respectively	Li et al. (2000)
Inhibition of lipopolysaccharide (LPS)-induced expression of the inducible nitric oxide synthase (iNOS) gene	$IC_{50} = 100$ μM	Lee et al. (2005)
Protective effect on oxidative DNA damage induced by Fenton reaction	Inhibits $OH^\bullet$-mediated damage to pBR322 plasmid DNA	Zhao et al. (2005)
CUPRAC assay	E‰ = 0.28 L/M/cm	Zaabat et al. (2011)
Inhibition of MDA generation	$IC_{50} = 3.82 \pm 0.5$ μM	Funes et al. (2009)

[a] Half-maximal scavenging concentration.
[b] 1 ORAC unit equals the net protection of fluorescein produced by 1 M Trolox.
[c] Absorbance at 700 nm.

leucosceptoside B) and subfractions that were obtained from *V. xanthophoeniceum* and consistently demonstrated moderate levels of inhibition (below 50%) at concentrations of 200 μg/mL towards acetylcholinesterase (47.94 ± 1.13%) and butyrylcholinesterase (39.19 ± 0.25%; Georgiev et al., 2011a). The memory-enhancing effects of verbascoside were also established in mice using a scopolamine-induced memory deficit model by passive avoidance and Morris water maze tests, suggesting the improvement of the function of the central cholinergic system (Lee et al., 2006b; Lin et al., 2012).

Oxidative damage that is caused by ROS has been considered to be one of the triggering factors of neurodegeneration and ageing and is involved in the pathologies of many neurodegenerative diseases (Santos et al., 2013; Schapira, 2006). Verbascoside has been revealed to exert robust antioxidant activities in many experimental models (Korkina, 2007) as shown in Table 1. Moreover, its plasma antioxidant capacity following oral ingestion and its capacity to enhance the endogenous antioxidant defences using malondialdehyde (MDA) generation, the FRAP value and SOD activity levels in several animal models have been fully reported and no acute or oral toxicity were observed up to 2000 mg/kg in rats (Funes et al., 2009; Quirantes-Piné et al., 2013a). These studies highlight the relationships of some structure-antioxidant activities and suggest that the four hydroxyls at the *ortho* position in the two aromatic rings of verbascoside contribute to its remarkable antioxidant activities (Zhou and Sadik, 2008). Moreover, its *iso* derivative (isoverbascoside) possesses a 2-fold stronger chelating activity compared with that of verbascoside, and phenolic hydroxyl groups play critical roles in its lipid peroxidation inhibitory and metal-chelating activities (Li et al., 1997).

The analgesic activity of isoverbascoside has been reported in two *in vivo* models of neuropathic pain (Isacchi et al., 2011), in which the phenylethanoid was applied orally (300–600 mg/kg) and intraperitoneally (100 mg/kg). Notably, both types of administration effectively reverted mechanically and chemically induced hyperalgesia. The anti-nociceptive effects of isoverbascoside (applied *per os* and topically) have been confirmed (Backhouse et al., 2008) in three different models of chemically and mechanically induced pain and compared with that of ibuprofen. In fact, the authors found that isoverbascoside and ibuprofen possess equal anti-nociceptive activities.

Verbascoside and cancer cells: chemotherapeutic versus chemopreventive approaches

Because the incidence of tumours has been steadily growing, there is an urgent need for preventive measures in addition to improved therapeutic approaches. For example, in the last two decades, natural plant-derived polyphenols (phenylethanoids, resveratrol, silybin, green tea polyphenols, flavonoids, anthocyanins, *etc.*) have been drawing particular interest as emerging active substances in dermatological/cosmeceutical compositions for the prevention, slowing, or reversion of skin tumourigenesis (chemoprevention). When they are chronically applied to the skin, they should not damage normal skin cells or negatively affect their functions but suppress tumorigenic cell transformation, inhibit tumour cell proliferation and activate tumour cell apoptosis. They are also reported to synergise with conventional anti-cancer therapies. There are a number of novel molecular and cellular targets including tumour stem cells, cellular senescence, epigenetic enzymes that are involved in carcinogenesis, epidermal growth factors, aryl hydrocarbon receptors and metabolic CYP1 subfamily enzymes, which have been reported to be positively affected by plant

polyphenols, which was recently reviewed (Korkina et al., 2013). Additionally, it has been assumed that verbascoside could exert anti-cancer, cytotoxic and anti-metastatic properties due to its estrogenic and anti-estrogenic functions (Korkina, 2007; Papoutsi et al., 2006).

Cancer cells are commonly characterised by the following three major disorders: a block of differentiation (the presence of non-differentiated cells), an inhibition of apoptosis and accelerated proliferation. The most recent publications have clearly shown that verbascoside may be considered to be a potent cancer chemopreventive/chemotherapeutic agent that is capable of inhibiting tumour cell proliferation and inducing their differentiation and apoptosis. Verbascoside has been reported to exhibit anti-proliferative activities towards some tumour cells *in vitro* (Wartenberg et al., 2003), to induce human gastric carcinoma cell differentiation and apoptosis by telomere–telomerase-cell cycle-dependent modulation (Zhang et al., 2002), and to repair DNA damage that is caused by oxidative stress (Li et al., 2000). In the well-described study by Lee et al. (2007), the mechanisms underlying the anti-proliferative effects of verbascoside on human promyelocytic leukaemia HL-60 cells have been revealed. Concentration of 30 μM induced a 50% inhibition of HL-60 proliferation, induced cell cycle arrest at the G0 to G1 transition by blocking cyclins D2, D3 and E in addition to the cyclin-dependent protein kinases CDK2 and CDK6, and, more importantly, induced tumour cell differentiation that was linked to specific biochemical activities and the expression levels of the CD14 cell surface antigen. These and other similar findings (Wartenberg et al., 2003; Zhang et al., 2002) provide convincing evidence that verbascoside, like all-*trans*-retinoic acid and vitamin D3, is a potent inducer of the terminal differentiation of leukaemia cells towards the monocyte/macrophage lineage. Hence, verbascoside has been proposed to be the investigational drug for the treatment of patients with myelo- and other types of leukaemias.

Excessive exposure to solar UVA and UVB radiation is widely considered to cause skin cancers, such as squamous cell carcinoma and basalioma. Direct UVB damage to skin cell DNA and UV-induced chronic skin inflammation, accelerated keratinocyte proliferation, inhibited apoptosis and immunosuppression seem to underlie the process of UV-induced carcinogenesis. Additionally, UVB induces cytochrome P450 subfamilies (CYP1A1 and CYP1B1) that are involved in the metabolic activation of organic pro-carcinogens and their ultimate conversions to carcinogens. Current efforts towards the chemoprevention of non-melanoma skin cancers encompass the search for natural non-toxic substances that are suitable for chronic topical application and able to hinder the carcinogenic effects of UV radiation. To date, with the exception of oral retinoids, no other natural or synthetic compound has been approved in humans, neither orally nor topically, as chemopreventive agents against the two prevalent UV-associated cutaneous non-melanoma malignancies. In accordance with recent publications, verbascoside may be a good candidate for skin cancer chemoprevention due to its prominent and long-lasting post-UV anti-inflammatory activities towards epidermal cells (Kostyuk et al., 2013).

The biotechnological production and (bio)synthesis of verbascoside

It is important to develop sustainable methods to produce valuable verbascoside for pharmaceutical applications. Plant *in vitro* technologies have been utilized for over a century. Additionally, plant cell/tissue culture has become increasingly attractive as cost-effective alternative to classical approaches for the sustainable mass production of plant-derived molecules (so-called 'green cell factories' concept) because of their numerous advantages. First, genetic modification in a contained system can readily be applied without the regulatory barriers that are associated with field-grown crops. Second, a cell/tissue culture system can be up-scaled in bioreactors with controllable production rates (Lim and Bowles, 2012). Further, plant cell/tissue culture is the only economically feasible way of producing some high-value metabolites from rare and/or threatened plants. The progress in this field has resulted in the mass production of several important metabolites (most notably paclitaxel, shikonin and berberine) by different companies (Georgiev et al., 2013). Early research attempts described the induction of a cell suspension culture of *S. vulgaris*, which was found to accumulate large amounts of verbascoside of up 16% on cell dry weight basis (Ellis, 1983). The improved production of verbascoside (2.3-fold) was observed when a cell suspension culture of *Cistanche salsa* was fed with a combination of phenylalanine, tyrosine and cucumber juice (a cheap source of caffeic acid; Liu et al., 2007). In a recent metabolomics study involving liquid chromatography–mass spectrometry (LC–MS), Guarnerio et al. (2012) observed that the exposure of an *Echinacea angustifolia* cell culture to light inhibited the rhamnosylation of caffeoyl phenylethanoid glycosides, thus detrimentally affecting the biosynthesis of verbascoside. Georgiev et al. (2011c) reported the successful up-scaling of its bioproduction by cell suspension cultures of devil's claw (*Harpagophytum procumbens*, Pedaliaceae) in a new bioreactor. The transfer from shaken flasks to the pulse-aerated column reactor resulted in 165.42 mg verbascoside/L/day, which is one of the highest productivity levels that has been reported to date. All of these studies demonstrate the feasibility of biotechnological production, although further up-scaling (Georgiev et al., 2013) is required for the viable commercial exploitation of this *in vitro* plant culture system.

Plant tissue/organ cultures are also attractive sources of verbascoside. Adventitious (normal) root cultures of *Castilleja tenuiflora* were grown in Gamborg's B5 medium that was supplemented with exogenous auxins (either 10 μM indole-3-acetic acid or 10 μM α-naphthaleneacetic acid). After 30 days of submerged cultivation, verbascoside reaches its peak of 14.62 mg/g dry root biomass (Gómez-Aguirre et al., 2012). Another attractive option for its bioproduction is hairy root cultures that are induced *via* an *Agrobacterium rhizogenes*-mediated genetic transformation, which has received increasing attention in recent years because they have relatively fast growth rates in hormone-free media, are genetically and biochemically stable and may possess similar secondary metabolite profiles as the plants from which they are generated (Georgiev et al., 2012). By transforming the stem explants of *P. tomentosa* with the *A. rhizogenes* strains LBA 9402 and A4, Wysokinska and Rozga (1998) obtained a hairy root clone that was capable of growing under submerged conditions and accumulating 9.5% verbascoside. Additionally an efficient protocol has been developed for the establishment of hairy root cultures of *V. xanthophoeniceum* using sonication-assisted *A. rhizogenes*-mediated transformation (Georgiev et al., 2011d). Ten days after inoculation with the *A. rhizogenes* ATCC 15834 suspension and 45 s of ultrasound exposure, hairy roots appeared on 75% of the *Verbascum* leaf explants. Moreover, the most vigorous *V. xanthophoeniceum* hairy root clones showed stable growth under submerged cultivation and accumulated high biomass amounts (13–14 g dry root mass/L). LC–MS metabolite profiling of the hairy roots revealed that verbascoside was the most abundant secondary metabolite, and its amounts were over 6 times higher than those in the mother plant tissues (Georgiev et al., 2011c). Clearly, plant *in vitro* systems (both dedifferentiated and differentiated) have enormous biosynthetic potentials and may therefore serve as attractive sources for the bioproduction of the pharmaceutically valuable compound verbascoside, although more detailed future research is needed.

In addition to the interest in verbascoside for medical purposes, its biosynthetic pathway remains to be fully elucidated. The early steps are known, but several downstream intermediates, key enzymes and their corresponding genes remain to be discovered. Current knowledge of the pathway, which is based on feeding experiments with stable isotope labelled precursors, is summarised in Fig. 4. Its biosynthesis begins with the generation of phenylalanine and tyrosine precursors by the shikimate pathway. The hydroxytyrosol moiety of verbascoside is biosynthesised from tyrosine either through tyramine and/or dopamine, whereas its caffeoyl moiety is synthesised from phenylalanine *via* a cinnamate pathway (Ellis, 1983). Dopamine is incorporated into verbascoside through oxidation to the corresponding aldehyde, reduction to the alcohol, and finally, β-glycosylation (Saimaru and Orihara, 2010). A study involving cell suspension cultures of *S. vulgaris* revealed

that the dihydroxy precursors, including DOPA and dopamine, are much less efficiently incorporated into verbascoside than the corresponding monohydroxy compounds (*i.e.*, tyramine, tyrosol and salidroside; Ellis, 1983). Although no intermediates leading from caffeic acid, salidroside, hydroxytyrosol, glucose, and rhamnose to verbascoside have been identified to date, and thus, no determined enzymatic steps are known, the proposed upstream pathway (Fig. 4) can be, in theory, engineered to boost production rates. Clearly, further information regarding verbascoside biosynthesis is needed to develop a viable biotechnological production process using metabolically engineered plant material. Obtaining this information will definitely require the robust identification of all of the intermediates, followed by the thorough characterisation of the enzymes and genes that are involved in the committed steps, as we as their regulation at cellular level.

The complete artificial synthesis of verbascoside has been achieved by Duynstee et al. (1999). In this study, a group from Leiden University (The Netherlands) reported the 15-step synthesis of verbascoside, resulting in an overall yield of 7.1%. Further, the physical and spectroscopic data of synthesised verbascoside were found to be identical to those that were reported for naturally occurring verbascoside.

Downstream processing of verbascoside

The development of cost-efficient technologies for the mass production of verbascoside requires that significant attention be paid to the downstream processing of verbascoside-containing (bio)mass. Efficient, convenient methods for its separation and purification from plant extracts have been developed (Han et al., 2012; Yue et al., 2013) involving the use of high-speed countercurrent chromatography (HSCCC). This technique generally eliminates irreversible adsorption, which is a common problem that occurs in column chromatography. By applying HSCCC in combination with macroporous resin column separation, Yue et al. (2013) succeeded in isolating and purifying five phenylethanoid glycosides, including forsythoside B, verbascoside, alyssonoside, isoverbascoside and leucosceptoside B from *Lamiophlomis rotata* (Benth.) Kudo. A two-phase solvent system that was composed of ethyl acetate/*n*-butanol/water (13:3:10, v/v/v) was used for the one-step HSCCC separation (4 h), which resulted in successful isolation of above-mentioned phenylethanoid glycosides at high purities (between 97.3% and 99.5%). In *Cistanches deserticola* Han et al. (2012) combined an enrichment step on a silica gel column with purification by preparative HSCCC (system: ethyl acetate/*n*-butanol/ethanol/water, 40:6:6:50, v/v/v/v) to isolate verbascoside, among other compounds, at purity levels exceeding 95% as determined by high performance liquid chromatography. Further, an ultrasound-assisted continuous approach for the direct enrichment of edible oils (olive, sunflower and soya) with the main phenols in olive leaves (*i.e.*, verbascoside, oleuropein, apigenin-7-glucoside and luteolin-7-glucoside) has been developed (Japón-Luján, et al., 2008). Under optimal conditions, only 20 min were necessary to enrich the edible oils for the above-mentioned bioactive molecules. The enrichment method is carried out at room temperature in an organic-solvent-free system, which ensures that the healthy properties of the edible oils improve along with their qualities.

Conclusion and perspectives

Fifty years after the discovery of verbascoside, very little is known about its biosynthetic pathway. Several key enzymes and the genes encoding them remain to be discovered. Thus, the improved understanding of its biosynthesis is required to identify means for boosting this process by metabolic engineering and subsequently for the development of efficient green cell/tissue factories for its mass production. Recent advances in transcriptomics and metabolomics platforms are likely to greatly facilitate such efforts.

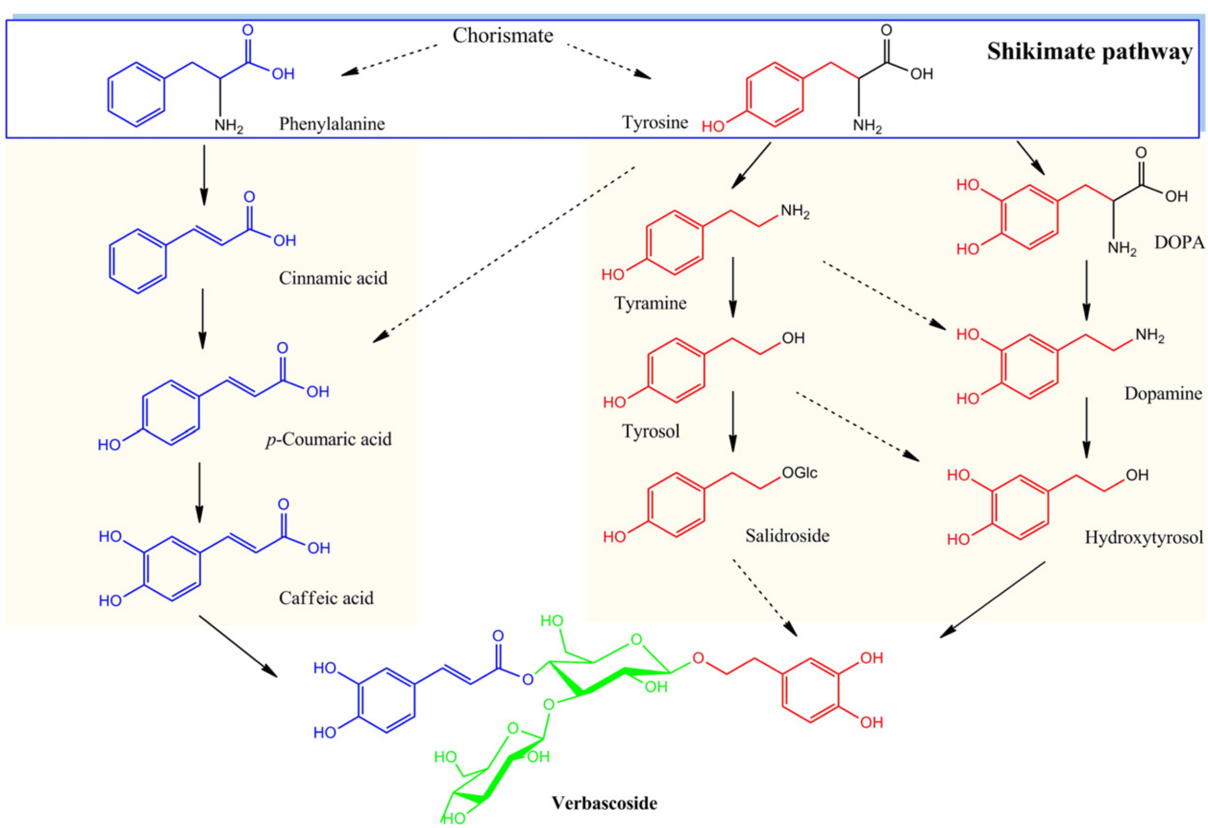

Fig. 4. Tentative pathway of verbascoside biosynthesis, that was based on feeding experiments with stable isotope labelled precursors (modified after Ellis, 1983; Saimaru and Orihara, 2010).

According to the data that is reported here, verbascoside exhibits neuroprotective effects through cholinergic, antioxidant and anti-inflammatory mechanisms and may be a promising candidate for neuroprotective applications. Its highly positive activities in the models of intestine inflammation provide insight regarding its feasibility in chronic inflammatory bowel disease. This compound shows great promise for the prevention and treatment of a variety of skin disorders, from immune-mediated chronic inflammatory disease to solar UV-induced cutaneous non-melanoma tumours. However, it should be noted that some of these activities occurred at relatively high concentrations of verbascoside and have been mainly observed in the *in vitro* or animal experiments. To date, reliable clinical data describing the health effects of verbascoside are limited and controversial; therefore, these studies should be considered with caution and more clinical trial on its efficacy and safety should be performed.

Despite the wealth of laboratory data that is available describing the anti-inflammatory effects of verbascoside following *in vitro* observations in addition to those involving animal models, many issues remain unresolved with respect to effective clinical applications. First, large-scale evidence-based human studies with specific therapeutic settings are necessary. The administration routes represent an additional crucial issue for clinical application. Different models have been used to demonstrate its protective effects following topical *vs* systemic application. To prevent tissue damage, oral administration is preferable due to the ease of intestinal *vs* cutaneous absorption and the lower risk of autooxidation of the phenol moiety. A promising perspective for *in vivo* effects is indeed envisaged in the administration to skin though appropriate stabilising means of delivery (Schmitt et al., 2009). However, at present, despite numerous *in vitro* studies demonstrating the free radical scavenging and anti-inflammatory properties of plant polyphenols, their low bioavailabilities and poor contributions to the total antioxidant capacities of plasma, the direct radical-scavenging properties *in vivo* remain uncertain. The indirect antioxidant activities of verbascoside due to the induction or/and activation of major endogenous antioxidant enzymes and inactivation of pro-oxidant enzymes could be envisaged. Another important issue is the extremely fast metabolism of verbascoside in the human body due to the multiple metabolic pathways that are involved to eliminate plant-derived toxins (Korkina et al., 2008). Further intensive studies are required to confirm the clinical potential of verbascoside, thereby enabling its acceptance as a therapeutic agent. It is also of interest for further chemical modifications because its structure offers an interesting scaffold (with various reactive sites) for combinatorial chemistry.

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